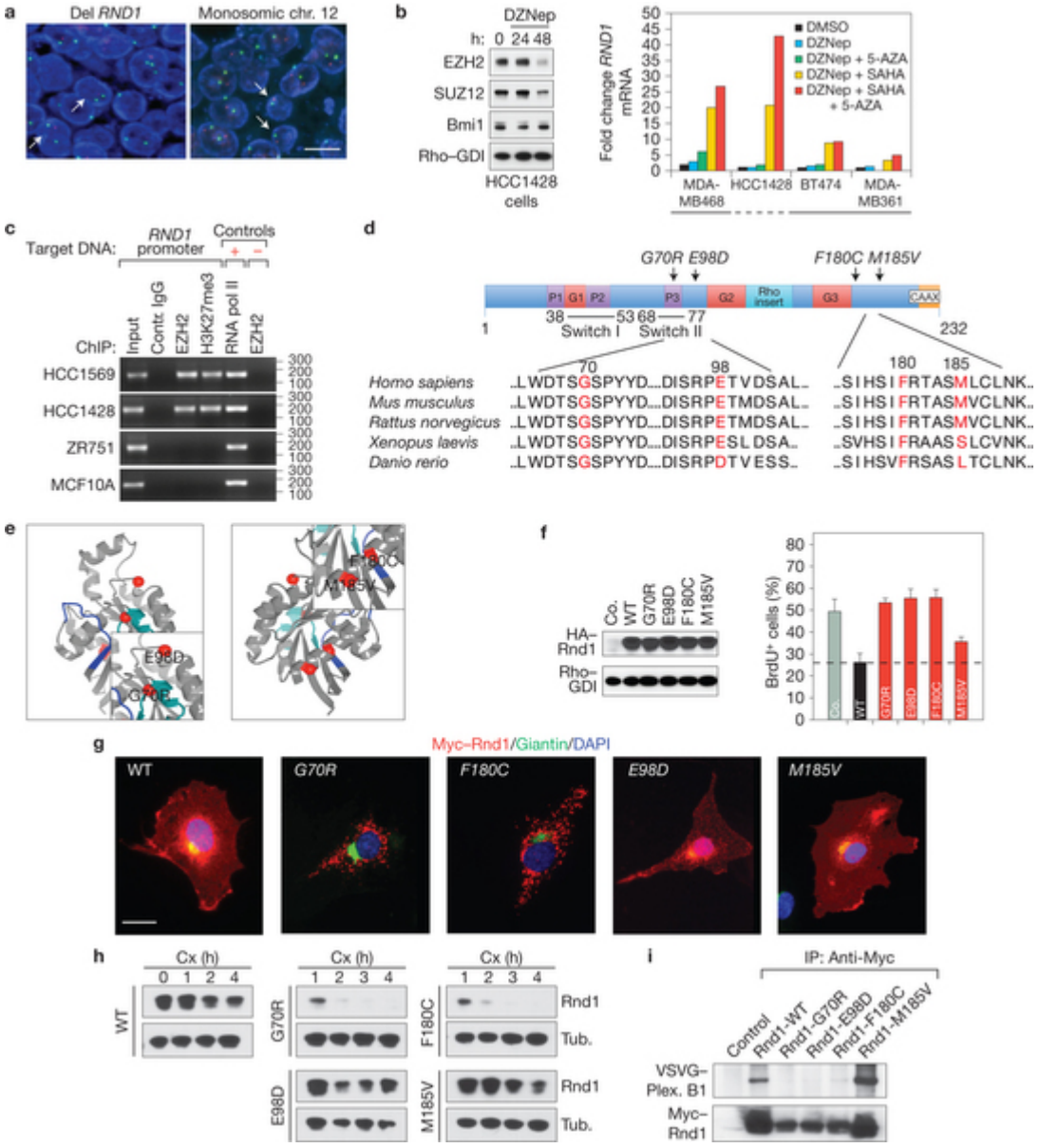
Figure 8: Genetic and epigenetic inactivation of *RND1* in human breast cancer.



(a) Representative images of breast cancer sections hybridized with a centromeric 12 chromosome (green) and locus-specific *RND1* (red, RP11-270J9) probe. Left panel: *RND1* deletion (single red dot); right panel: 12 chr. monosomic deletion. Scale bar, 20 µm. (b) HCC1428 cells treated with DZNep (5 µM) were immunoblotted with the antibodies indicated (left). Human breast cancer cell lines were treated with DZNep in combination with either 5-Aza (10 µM), SAHA (5 µM), or both and *Rnd1* transcript was assessed by qPCR. Data are from one experiment shown as averages of three technical replicates (the experiment was repeated 2 times). (c) ChIP assay of the *RND1* promoter with antibodies against EZH2, H3K27me3, or control RNA pol II and IgG, as indicated. Sequences from the *GAPDH* promoter and a *RND1* intron were used as positive (+) and negative (–) controls, respectively. (d) Schematic representation showing the domain organization of Rnd1. Arrows point to the position of tumour-derived mutations. The amino acid sequences surrounding mutated residues (red) from various species are aligned below. (e) Crystal structure of Rnd1 and insets show magnifications of relevant regions. Tumour-derived mutation residues are depicted as red balls. Switch I and II segments are depicted in blue and cyan, respectively. (f) MCF-10A cells expressing HA-tagged wild-type or mutant RND1 proteins or empty vector (Co.) were deprived of growth factors and subjected to BrdU incorporation assay. Data are averages of *n* = 3 technical replicates from one experiment (the experiment was repeated 2 times). Error bars are s.d. (top).

Immunoblotting shows the expression of HA–Rnd1 (bottom). (g) HUVEC cells were transfected with Myc-tagged forms of Rnd1 and subjected to immunofluorescent staining with anti-Myc (red), giantin (green) and DAPI (blue). Scale bar, 15 μM. (h) 293T cells transfected with Myc-tagged Rnd1 were treated with cycloheximide (Cx; 75 μg ml^{-1}) and immunoblotted with anti-Myc and anti-tubulin. (i) 293T cells transfected with the indicated Myc-tagged Rnd1 or empty vector in combination with VSVG–Plexin B1 were immunoprecipitated with anti-Myc, followed by immunoblotting with anti-VSVG or anti-Myc. b,c,f show one representative experiment out of two performed, whereas g–i show one representative experiment out of three performed. Biological replicates yielded similar results. Source data are provided in Supplementary Table8. Uncropped images of blots are shown in Supplementary Fig. 9.